SUMMARY AND CONCLUSIONS

India is basically an agricultural country, where agriculture is a gamble in the monsoon. The vagaries of the monsoon are well known. Naturally the failure of monsoon is disastrous to the life and economy of the country. Therefore it is important to cultivate crop plants which can be adapted to these inhospitable agroclimatic conditions.

In addition to above mentioned conditions, more than 0.714 million hectares of land is saline, saline alkali or alkali. Hence the problem of water stress coupled with salinity becomes very significant.

Gujarat lies between 20° North latitude and 24° North latitude. A large part of the state lies in the tropical region. It has a monsoon climate.

In agriculture the problem of drought and salinity becomes more acute and severe every year as the non-saline soils and non-saline water are intensively and extensively exploited.

Plants species differ in their tolerance to different types of water stress. Also crop that are highly tolerant at one growth stage become sensitive during another stage. Hence, to find out the sensitive and tolerant stages,
early stages of germination (Laboratory condition) were chosen for this work.

To study the resistance physiology, in this work, studies on behaviour of seedlings and associated biochemical changes under the effects of water and salinity stress and desiccation were undertaken. The crop plants i.e. *Medicago sativa* Linn. (Anand - 2) and *Phaseolus aconitifolius* Jacq. (Kalol - Deshi) were selected for this work.

*Medicago sativa* Linn. is a most important Leguminous fodder crop in the world. This plant extensively grown throughout the Gujarat State and is used as a green fodder all over Gujarat.

*Phaseolus aconitifolius* Jacq. is a Kharif crop, grown everywhere in Gujarat. It is used as a split pulse. The stalks and leaves are also used as fodder for cattle.

Numerous short term studies in the last few decades have detailed a variety on physiological and metabolic changes in plants caused by different stress, but a detailed study of a single species would be great help for better understanding of its metabolic and physiological conditions altered by water and salt stress.
For experimental convenience the whole investigation was divided into two experiments during this work.

Experiment - 1: Effect of water stress and salinity stress on extension growth and biochemical changes during germination of (A) *Medicago sativa* Linn. (Anand-2) and (B) *Phaseolus aconitifolius* - Jacq. (Kalol-Deshi).

Experiment - 2: Effect of desiccation treatment on biochemical changes during germination hours of (A) *Medicago sativa* Linn. (Anand - 2) and (B) *Phaseolus aconitifolius* - Jacq. (Kalol - Deshi).

Experiment - 1

The seeds of both the plants were germinated in the following manner.

The seeds of both the plants were germinated in sterilized Petridishes, lined with sterilized filter paper in adequate (i.e. 3.5 ml in plant (A) and 5.0 ml. in plant (B)) and restricted (i.e. 1.7 ml. in plant (A) and 2.5 ml. in plant (B)) media of distilled water and salinity (0.1 M NaCl) separately.

At an interval of 24 hours of germination, under water stress and salinity stress the present data have been recorded according to the standard methods.
The data of water stress and salinity stress have been compared with the data of controlled (i.e. adequate distilled water) seedlings and the results were obtained during germination. The conclusions drawn from these are as under:

1. The water deficit could be simulated by germinating seedlings in restricted media of distilled water and salt as well as by subjecting them to desiccation treatment.

2. The response of two seeds to the same stress is different in certain cases. Restricted media of salinity and distilled water caused delay in germination.

3. Restricted media of distilled water and both the quantities of salt solution retarded root and shoot extension growth, fresh weight and dry weight of both organs. Restricted supply of salinity retarded root/shoot length more than the adequate supply of salinity and restricted supply of distilled water.

The delay in the germination is due to delay in initiation of mitotic activity under water stress condition. The reduction in extension growth of seedling under stress condition is due to less uptake of water.
4. Differential effects of salinity on growth rates might be due to specific ion toxicities. The effects of specific ions make definite contribution to the overall effect of salinity in plants.

5. During stress condition, seedlings try to adjust their osmotic concentration, either by accumulation of organic solutes by the cells specially hexose and disaccharides or by adjustment of the moisture content.

6. Total sugar (TS) content and Non-reducing (NRS) content increased under drought condition in both (A) and (B) seedlings. Accumulation of non-reducing content may also be considered as an adaptation on the part of seedlings against stress condition. NRS can serve as a reservoir or pool after wards they can be converted to RS as and when required to provide energy. Secondly they may help seedling in osmotic adjustment, thereby preventing osmotic loss of H₂O through exosmosis. The sugars were utilized only for osmotic adjustment, interconversion into other cellular polysaccharide could not occur, thus there was a lesser availability of those cellular constituents probably for cellulose, which results into retardation of extension growth or elongation of root and shoot.
The decreased sugars content at late hours of germination under drought in (A) seedling may provide Alpha - Ketoglutarate and NADP(H) for proline synthesis which is known to increase relative hydration under water stress. This is supported by accumulation of proline under drought condition in presents study.

7. Amylase activity in (B) seedling exhibited varied responses with respect to time, organ and quantities of germinating media.

The stimulation in embryonal amylase was observed under adequate media of salinity. This may be explained as the theory of stress physiology that hydrolytic enzymes increased wherever there is a stress. Whereas in cotyledons amylase activity was less under stress condition. This may be due to reduced availability of water to the cotyledons for enzyme synthesis.

8. There was lesser cotyledons starch upto 48 hours in the stressed seedlings of (B) Plant. It is well documented that water stress causes starch depletion due to hydrolysis by the enzyme amylase to provide sugars. There was higher amylase activity in the embryo of (B) seedling at initial hours of
germination as compared to the cotyledons, despite the lower starch content which suggests that the embryo is the major seat of enzyme activity.

9. Invertase enzyme remained inactive at initial hours of germination, under deficit of water as the high activity was found in adequately watered seedlings. Whereas at later hours of germination invertase activity increased in (A) seedling and cotyledon of (B) seedling under low moisture level suggests that the protection is afforded at later stages of germination by increased RS content under unfavourable conditions.

10. A high degree of resistance to drought was largely due to seedling ability to renew protein during the stress period. In present study during salinity stress higher level of protein was observed in (A) seedling. Whereas the response of (B) seedling was different. In embryo salt stress fluctuated protein content in embryo and decreased them in cotyledons as compared to controlled seedlings. Protein is hydrolysed in stress condition yielding a mixture of amino-acids, amides and simple peptides. Due to this process, amino acids formation was observed at early hours of germination in (A) seedling, at late hours these amino acids decreased due to their
utilization in protein synthesis for developing embryo. The decreased protein content reduce to the higher action of protease in saline condition. The restricted supply of salinity caused water as well as salt stress, which together might have changed the pattern of protein synthesis.

11. The response of different stages was different for amino acids accumulation in reference to salinity in (A) and (B) seedlings. A considerable varietal difference in the accumulation of free amino acids in response to salinization is well documented. A striking accumulation of the free amino-acids occurred at same stages of germination in response to changing osmotic potentials in their external environment by osmotic adjustment of their cellular contents.

12. Radio traces experiments (Cecil, 1977) have inferred that the synthesis of proline from glutamic acid is stimulated by water stress. High level of proline content and its accumulation under stress, during present study might have acted as storage compound for carbon and nitrogen. Accumulation of proline and stress tolerance have positive correlation. The proline accumulated during water stress, might have protected the structure and
functions of protein being excluded from the protein hydration sphere. In (A) seedling during germination under adequate and restricted media of salinity, proline lowered down up to 72 hours and accumulated after initial decrease respectively. The variations in proline content of salt stressed Medicago sativa L. may be symptomatic of the deleterious influence of salt stress, only regards to its reduced accumulation at early hours of germination. There was more proline in the salt treated seedlings that survived in the unfavourable condition up to 96 hours, after germination when observed on the 48th hours of germination. This could mean that the Medicago sativa L. seedlings survived because they built up the critical proline level needed for their survival. The same is also true for Phaseolus aconitifolius Jacq.

13. Soluble protein (Enzyme protein) content increased under water stress condition. Synthesis of some specific enzymes increased under water stress condition, in (A) seedling. Where as in (B) seedling, increase or decrease in soluble protein content varied with the time of germination and quantities of germination media of salinity.
In (A) seedling RNA synthesis was inhibited under drought condition. Whereas in embryo of (B) seedling the synthesis of RNA was increased as compared in that under normal moisture level. In both the seedlings RNase activity was fluctuated under drought condition. As RNase activity is measured in juvenile phase, it became a synthesizing enzyme rather than a hydrolysing enzyme, as it worked under the phase of drought hardness as a result it increased RNA content at juvenile phase.

Salinity reduced RNA content RNA content at early hours and increased it at late hours of germination in (A) seedling. The synthesis of RNA in embryo of (B) seedling was inhibited under adequate salinity while it fluctuated under restricted salinity as compared to that under controlled condition. The increase or decrease in level of RNA depends upon RNase activity or it depends upon the synthesis of RNA under unfavourable condition.

RNase activity was inhibited in (A) seedling at early hours of germination and stimulated at late hours of germination under salinity as compared to that under normal condition. Stimulation in RNase activity at certain germination stages with increased RNA content might have changed RNA composition and new RNA
species might have been formed. Base composition of nucleic acid is known to be changed by water stress. In embryo of (B) seedling enhanced RNase activity was observed in salt treated seedling. In cotyledons adequate and restricted media of salinity acted differently on RNase. It is well known that enzyme activity depends on the amount level of its substrate.

15. Drought treatment in (A) seedling - caused no effect on DNA, in younger seedling where as it reduced DNA content in older seedling. The same is true for (B) seedling. The reduction in DNA content might have occurred due to inhibition in its synthesis which further affected the growth of seedling.

Adequate salinity increased DNA content in (A) and (B) seedlings. Restricted salinity supply lowered down by restricted media of salinity. In cotyledon of (B) seedling, DNA was decreased. This suggests that the breakdown of DNA from cotyledons supplies the basic substance for synthesis of protein to supply for growth and development of embryo as it gets necessary substances from the reserve food.

16. Phenolic compound content increased under drought condition in (A) seedling and decreased under salt
stress, in (A) seedling, this content decreased under drought condition and under saline condition increased up to 48 hours and decreased thereafter. A major role has been assigned to phenolic acids as growth regulators of plant growth and development. The inhibition in growth by phenolic compounds may be through suppression of activities of IAA, GA and cytokinins.

Under salinity phenolic compound comparatively reduced in (A) seedling. Restricted as well as adequate salinity increased phenolic compound in (B) embryo, at early hours of germination and inhibited synthesis at late hours of germination or content may be increased due to low utilization, under saline condition.

17. The response of (A) and (B) seedling to drought was different at different germination hours for oxidizing enzyme catalase. The increased catalase activity might have oxidized \( H_2O_2 \), thus prevented any peroxidative damage. In cotyledon of (B) seedling decreased catalase activity was observed, under stress condition. Due to lower respiration rate in cotyledon, lesser energy may be available to seedling which therefore arrested the growth under water stress.
Catalase and peroxidase play the protective role against oxygen toxicity in plant cells. The higher levels of peroxidase and catalase may be essential for seedlings in saline condition to overcome the adverse condition. The unique response to these enzymes, to salinity may be regarded as possible mechanism facilitating survival during adverse condition rather than growth maintenance. The decline in catalase activity at 96 hours due to production of an inhibitor, or inhibition of enzyme synthesis or change in assembly of enzyme subunits. Inhibition of enzyme synthesis was the main reason for reduction in catalase activity under saline condition.

10. Under drought condition, with (A) seedling, peroxidase activity reduced at early hours of germination and increased at late hours of germination. Peroxidase activity is associated with active differentiation and cell regulation. Initially low peroxidase activity can be correlated with poor differentiation of embryo axis in (B) seedling. Stimulation in enzymic activity at late hours pointed out that, respiratory and oxidative processes were at higher rate. Peroxidase activity rises whenever there is a stress.
Activated oxygen in the form of free radicals such as superoxide and the hydroxyl radical or as \( \text{H}_2\text{O}_2 \) is very toxic to the plant cells. Both the media of salinity stimulated peroxidase activity in (A) and (B) seedling with the advancement of germination. As the growth advanced, the growing embryo needed energy, a part of which could be produced by peroxidase activity eliminating toxic \( \text{H}_2\text{O}_2 \), which was the product of higher respiratory process as the growth advanced. From the data of growth and peroxidase, it can be inferred that, salinity might be inhibiting the growth via increase in peroxidase activity.
EXPERIMENT : 2

Graded and surface sterilized seeds of both the (A) and (B) plants were germinated in adequate (i.e. 3.5 ml. in (A) plant and 5.0 ml in (B) plant) media of glass-distilled water as described in Experiment -1 and were subjected to desiccation chamber for a given period (i.e. 2-day and 4-day of time). They were then revived by keeping them on the moisture sterilized sand in Petridish. The estimations mentioned in Experiment -1 were carried out (i) before the initiation of the desiccation treatment, (ii) at the end of desiccation period and (iii) after revival.

The data of desiccated and revived seedlings have been compared with the data of controlled seedlings, (i.e. before the initiation of the desiccation treatment) and the results and conclusions drawn from this are as under:

1. After desiccation treatment, the germinated seedlings were transferred to 100 % relative humidity for slow rehydration in order to minimize damage to cells from hydrational forces.

The present author frequently noted the appearance of lateral roots after the primary radical had been killed by severe - 4 - days - desiccation. Thus the
desiccated seeds were able to continue growth and development after rehydration.

2. During desiccation, in (A) seedlings, TS, NRS and RS content increased as compared to undesiccated seedlings. Increased sugars acted as a protecting solvent against dehydration. Rise in sugars content can be considered as a biomechanism to survive the stress condition. Soluble sugars especially sucrose acts a protective role and gives tolerance against desiccation. 2-days desiccation treatment found to be more beneficial for total sugars in embryo of (B) seedling, where as in cotyledon both the desiccation treatments increased sugars.

3. Enhanced amylase activity in the desiccated seedlings can be interpreted as increased synthesis of amylase enzyme.

4. Deppletion of starch during desiccation, indicated that the water stress and desiccation cause hydrolysis by enzyme amylase. Rewatering also stimulated amylase activity. Starch content and amylase activity had direct relation in Phaseolus aconitifolius Jacq. Thus the hydrolytic conversion of starch results into the formation of sugars.
5. In (A) seedling desiccation treatment greatly increased invertase activity in younger seedlings but depressed it sharply in older seedlings. Severe desiccation caused more stimulation. The same is true for (B) seedling. Depressed invertase activity allowed accumulation of sucrose which had a protective role as discussed earlier. The protection was afforded at later stages of germination by this sugar under unfavourable condition.

6. Desiccated and revived (A) seedlings showed more protein content than undesiccated seedlings where as in (B) seedling, desiccation treatment decreased total protein content. (A) seedling might have tolerance power against desiccation while (B) seedling was sensitive to desiccation with protein metabolism. Moisture stress usually decreases plant protein content. Under desiccation condition, break down of protein might have occurred into soluble nitrogen, amides and soluble protein.

7. In (A) seedling protease activity was more in younger desiccated seedlings while it was less in older desiccated seedling. While in (B) seedling enzymic activity was fluctuated during desiccation. Stimulated protease activity might have resulted into synthesis of new proteins as the protein content was
high in desiccated seedlings. A high degree of resistance to drought is largely due to ability of plants to renew protein, during the stress period.

8. Accumulation of amino acids was observed during desiccation. The accumulated amino acids may again used to renew protein after revivals. Which increased protein content in revived seedling.

9. DNA content increased during desiccation with (A) seedling where as the same treatment reduced DNA content while in revived seedling DNA content increased. The accumulation of intermediate product of nucleic acid metabolism might have occurred during desiccation.

10. Less RNA content and less RNase activity at some stage whereas more RNA content and more RNase activity at another stage of germination was observed with (A) and (B) seedling. It suggests that at some stages RNase acts as a hydrolytic enzyme while at some other stages it acts as a synthetic enzyme.

11. Phenolic compound content accumulated during desiccation suggests stimulation at synthetic side or less utilization, which reduced in desiccated seedling of late germination period. Which suggest
the utilization of previously accumulated phenolic compounds at late hours.

12. During desiccation, catabolic processes are surpass the anabolic processes, the growth of the seedling comes to a stand still. This was quite evident from the fact that in the present study, during the period of desiccation no further growth of the seedling is observed. During 2-day and 4-day desiccation the seedling remains at the same stage at which it was desiccated.

To summarise, the present study elucidates that in seeds germinated under water stress condition, there is a general breakdown of essential metabolites like RNA, DNA protein. As well as decreased activity of enzymes associated with growth, consequently reduces extension growth of the seedling.

The plants, subjected to water stress when revived under optimal condition of moisture, causes the resynthesis of lost metabolites and reactivation of enzyme associated with growth. Thus the environment is the cause and metabolism is the effect.

The variations in the results of metabolic activities of a seedling, under stress condition, is most probably due to differences in the degree of
resistance (tolerance power) or dehydration at different phases of seedling growth as well as in different organs of the seedling under varying environmental conditions.